SUPPLEMENTAL MATERIAL

Xanthine Oxidase Drives Hemolysis and Vascular Malfunction in Sickle Cell Disease

Heidi M. Schmidt[1], Katherine C. Wood[2], Sara E. Lewis[3], Scott A. Hahn[2], Xena M. Williams[3], Brenda McMahon[2], Jeffrey J. Baust[2], Shuai Yuan[2], Timothy N. Bachman[2], Yekai Wang[4,5], Joo-Yeun Oh[6], Samit Ghosh[2,7], Solomon F. Ofori-Acquah[2,7,8], Jeffrey D. Lebensburger[9], Rakesh P. Patel[6,10], Jianhai Du[4,5], Dario A Vitturi[1,2], Eric E. Kelley[3], and Adam C. Straub[1,2]

[1]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, Pennsylvania; [3]Department of Physiology and Pharmacology, Health Sciences Center, West Virginia University, Morgantown, WV; [4]Department of Ophthalmology, West Virginia University, Morgantown, WV; [5]Department of Biochemistry, West Virginia University, Morgantown, WV; [6]Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham, AL; [7]Division of Hematology/Oncology, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA; [8]School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana; [9]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL; [10]Department of Pathology, University of Alabama at Birmingham, Birmingham, AL

SUPPLEMENTAL METHODS

Coumarin Boronic Acid (CBA) Assay

Oxidant load (H_2O_2, $O{=}NOO^-$, and HClO) was measured using coumarin boronic acid (CBA) as previously published with some modifications.[1] Plasma samples were collected and stored at -80ºC. To preserve oxidant production in plasma samples, sodium azide (20 mM) was supplemented to inhibit enzyme activity immediately upon thawing the plasma. CBA probe (Cayman, 14051) was prepared in the assay buffer (0.01% BSA in HBSS) and mixed with plasma samples at 0.5 mM final concentration. Kinetic fluorescence measurement was performed at 350/450 nm, 37 °C. The amount of oxidant load was quantified by the production rate of the fluorescent product in the log phase and expressed as fold change.

Pulmonary, Mesenteric, and Thoracodorsal (TDA) Ex-Vivo Two-Pin Wire Myography

Myography experiments were performed similarly to those previously described by our lab.[2] Briefly, mice were euthanized by heart puncture, following right heart catheterization (see below). Pulmonary, mesenteric, and TDA arteries were rapidly excised, placed in room temperature physiological salt solution (PSS), cleaned of fat, cut into 2 mm rings fitted with two 25 µm wires, and placed on a wire myograph (DMT 620M) filled with PSS containing (in mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, KH_2PO_4 1.18, D-glucose 5.5, $NaHCO_3$ 25, EDTA 0.027, $CaCl_2$ 2.5, pH 7.4 when bubbled with 95% O_2 and 5% CO_2 at 37ºC. Vessels were allowed to rest for 30 minutes in PSS buffer. Vessels were then incrementally stretched to a tension equivalent to 80 mmHg. To constrict the arteries and determine viability, 60 mM potassium (K) in PSS (KPSS) was added for 5 minutes. Vessels were washed (3x) and rested in PSS for 30 minutes. For vessels isolated from mice treated with febuxostat, 10 µM febuxostat was added to the PSS following the washes. For pulmonary arteries, vasoconstriction was induced with increasing doses of the constrictor prostaglandin F2α (PGF) (10^{-7}-10^{-5} M, Tocris #4214) at 4-minute intervals until the vessels reached maximum constriction. For mesenteric and TDA arteries vasoconstriction was induced with increasing doses of the constrictor U46619 ($10^{-7.5}$-$10^{-6.5}$ M, Cayman Chemicals) at 4-minute intervals until the vessels reached maximum constriction. Pulmonary, mesenteric, and TDA arteries were then treated with increasing doses of the vasodilator acetylcholine (10^{-8}-10^{-5} M, Sigma) at 3-minute intervals. Finally, vessels were treated with Ca^{2+} free PSS containing 100 µM SNP to determine maximal dilation. Data was collected using Lab Chart Software (AD Instruments) and normalized to the change in maximum constriction or maximum dilation to determine the percent relaxation of vessels.

Closed Chest Right Ventricle (RV) Micro-Catheterization

Micro-catheterization was performed as previously described.[3, 4] Briefly, mice were anesthetized with an intraperitoneal injection of etomidate/urethane (9/1.1 mg/kg, Butler Schein). Body temperature was regulated with a heating pad and heating lamp. While in the supine position, an incision was made between the sternohyoid and sternomastoid muscles of the right neck. Catheterization of an isolated 20 mm section of the external jugular vein was done using two 6-0 silk sutures by tying a surgical knot at the cranial end

of the external jugular vein and the other looped lightly on the same vein by the heart. A small incision was made in the vein and a 1.2F micro pressure-volume (PV) catheter was inserted beyond the suture by the heart which was then tightened to prevent bleeding. The catheter was further advanced until it passed through the right atrium and into the right ventricle. Pressure measurements were given five minutes to stabilize before beginning recording. The PV catheter was removed and the external jugular was ligated to prevent further bleeding. Pressure waveforms were saved and analyzed using IOX2 Software (EMKA Technologies; Falls Church, VA) and MATLAB (Mathworks, Natick, MA, USA).

Echocardiogram

Echocardiograms were performed by the University of Pittsburgh Rodent Ultrasonography Core. Mice were anesthetized with 3% isoflurane (maintained with 1.5% isoflurane) and body temperature was maintained with a heating pad. Images of the right and left ventricles were acquired using a Vevo 3100 imaging system and the VisualSonic MX400 (20-46 MHz, 50 μm axial resolution) linear array transducer (FUJIFILM VisualSonics, Toronto, Canada). Heart rate was maintained between 400-500 bpm by adjusting the isoflurane concentration between 1-2%. Image acquisition and analysis was done as previously described.[5]

Trichrome Staining

Trichrome staining was performed as previously described.[6] Tissues were stained using the Masson's Trichrome Kit (Thermo Fisher) in accordance with the manufacturer's guidelines. Sections were imaged using the TissueGnostics Microscope with a 20x objective and stitched using NIS Elements Software (Snake Stich, 8% overlap). Quantification of trichrome positive/fibrotic area was done using FIJI software.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure I. Characterization of chimeric SCD mouse model treated with febuxostat. A) Experimental design. **B)** Bone marrow engraftment evaluated by hemoglobin electrophoresis at 0, 3, and 10 weeks post-engraftment. Eighty percent was used as the threshold for adequate engraftment. Values are mean ± SEM using a one-way ANOVA with Sidak's multiple comparisons test. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. **C)** LC/MS-MS was used for purine metabolite analysis of hypoxanthine, xanthine, and urate. **D)** Febuxostat treatment decreased body weight of AA mice. Values are mean ± SEM using an unpaired Student's t test unless otherwise noted. $^{+}$Values are mean ± SEM using an unpaired Student's t test with Welch's correction. **E)** Spleen weight was normalized to tibia length. Values are mean ± SEM using a one-way ANOVA with Sidak's multiple comparisons test. ****P<0.0001. WT, wild type; febux, febuxostat; HypoX, hypoxanthine; SCD, sickle cell disease.

Supplemental Figure II. Evaluation of hemolysis in AA and SS mice treated with febuxostat. A) Experimental design. UV-visible spectral deconvolution was used to measure **B)** MetHb and **C)** OxyHb after 10 weeks of treatment. Values are mean ± SEM using a one-way ANOVA with Sidak's multiple comparisons test. *P<0.05, **P<0.01. UV-visible spectral deconvolution was used to measure **D)** plasma cell free hemoglobin, a combination of MetHb and OxyHb, and **E)** cell free hemin after 10 weeks of treatment. An ELISA was used to measure plasma **F)** haptoglobin and **G)** hemopexin concentration after 10 weeks of treatment. Values are mean ± SEM using an unpaired Student's t test. WT, wild type; febux, febuxostat; MetHb, methemoglobin; OxyHb, oxyhemoglobin; ELISA, enzyme linked immunosorbent assay.

Supplemental Figure III. Ex-vivo wire myography of AA mice treated with febuxostat. A) Ex vivo wire myography was used to asses vasoreactivity of pulmonary, mesenteric, and TDA arteries. **B)** Pulmonary, **C)** mesenteric, and **D)** TDA constriction was measured by normalizing to maximum KCl response. Values are mean ± SEM using an unpaired Student's t test unless otherwise noted. +Values are mean ± SEM using an unpaired Student's t test with Welch's correction. An acetylcholine dose response was used to measure dilation of E) pulmonary (AA n=12, AA + Febux. n=4), F) mesenteric (AA n=6, AA + Febux. n=4), and G) TDA (AA n=6, AA + Febux. n=4) arteries. Values are mean ± SEM using a two-way ANOVA with Sidak's multiple comparisons test. TDA, thoracodorsal; KPSS, potassium physiological salt solution; PSS, physiological salt solution; febux, febuxostat; max, maximum.

Supplemental Figure IV. XO inhibition did not affect the cardiac fibrosis observed in SS sickle mice. A) Experimental design. **B)** RV weight and **C)** LV + septum weight were normalized to tibia length. **D)** Trichrome staining of RV (top) and LV (bottom) heart sections. Scale bar = 100 μm. Quantification of **E)** RV and **F)** LV trichrome staining using FIJI software. Values are mean ± SEM using an unpaired Student's t test unless otherwise noted. #Values are mean ± SEM using a Mann-Whitney test. WT, wild type; febux, febuxostat; RV, right ventricle; LV, left ventricle; XO, xanthine oxidase.

Supplemental Figure V. Characterization and evaluation of hemolysis in hepatocyte-specific XO KO mice. A) Experimental design. **B)** Bone marrow engraftment evaluated by hemoglobin electrophoresis at 0, 3, and 10 weeks post-engraftment. Eighty percent was used as the threshold for adequate engraftment. **C)** LC/MS-MS was used for purine metabolite analysis of hypoxanthine, xanthine, and urate. **D)** Hepatocyte-specific XO KO did not alter body weight of HXdh-/- mice. UV-visible spectral deconvolution was used to measure **E)** plasma MetHb and OxyHb and **F)** cell free hemin 10 weeks post-engraftment. **G)** An ELISA was used to measure plasma hemopexin concentration after 10 weeks of treatment. Values are mean ± SEM using an unpaired Student's t test unless otherwise noted. [#]Values are mean ± SEM using a Mann-Whitney test. [+]Values are mean ± SEM using an unpaired Student's t test with Welch's correction. XDH, xanthine dehydrogenase; HypoX, hypoxanthine; MetHb, methemoglobin; OxyHb, oxyhemoglobin; XO, xanthine oxidase; KO, knockout; ELISA, enzyme linked immunosorbent assay.

Supplemental Figure VI. Evaluation of vasoreactivity and cardiac function of H*Xdh*$^{-/-}$ mice. A) Experimental design. **B)** Pulmonary, **C)** mesenteric, and **D)** TDA constriction was measured by normalizing to maximum KCl response. Values are mean ± SEM using an unpaired Student's t test. An acetylcholine dose response was used to measure dilation of E) mesenteric (*Xdh*$^{fl/fl}$ n=8, *Xdh*$^{-/-}$ n=7), and F) TDA (*Xdh*$^{fl/fl}$ n=8, *Xdh*$^{-/-}$ n=6) arteries. Values are mean ± SEM using a two-way ANOVA with Sidak's multiple comparisons test. **G)** RV weight and **H)** LV + septum weight was normalized to tibia length. Values are mean ± SEM using an unpaired Student's t test. XDH, xanthine dehydrogenase; max, maximum; TDA, thoracodorsal; RV, right ventricle; LV, left ventricle.

SUPPLEMENTAL REFERENCES

1. DeVallance E, Branyan KW, Lemaster KC, Anderson R, Marshall KL, Olfert IM, Smith DM, Kelley EE, Bryner RW, Frisbee JC and Chantler PD. Exercise training prevents the perivascular adipose tissue-induced aortic dysfunction with metabolic syndrome. *Redox Biol*. 2019;26:101285.

2. Rahaman MM, Nguyen AT, Miller MP, Hahn SA, Sparacino-Watkins C, Jobbagy S, Carew NT, Cantu-Medellin N, Wood KC, Baty CJ, Schopfer FJ, Kelley EE, Gladwin MT, Martin E and Straub AC. Cytochrome b5 Reductase 3 Modulates Soluble Guanylate Cyclase Redox State and cGMP Signaling. *Circ Res*. 2017;121:137-148.

3. Potoka KP, Wood KC, Baust JJ, Bueno M, Hahn SA, Vanderpool RR, Bachman T, Mallampalli GM, Osei-Hwedieh DO, Schrott V, Sun B, Bullock GC, Becker-Pelster EM, Wittwer M, Stampfuss J, Mathar I, Stasch JP, Truebel H, Sandner P, Mora AL, Straub AC and Gladwin MT. Nitric Oxide-Independent Soluble Guanylate Cyclase Activation Improves Vascular Function and Cardiac Remodeling in Sickle Cell Disease. *Am J Respir Cell Mol Biol*. 2018;58:636-647.

4. Wood KC, Durgin BG, Schmidt HM, Hahn SA, Baust JJ, Bachman T, Vitturi DA, Ghosh S, Ofori-Acquah SF, Mora AL, Gladwin MT and Straub AC. Smooth muscle cytochrome b5 reductase 3 deficiency accelerates pulmonary hypertension development in sickle cell mice. *Blood Adv*. 2019;3:4104-4116.

5. Rutledge C, Cater G, McMahon B, Guo L, Nouraie SM, Wu Y, Villanueva F and Kaufman BA. Commercial 4-dimensional echocardiography for murine heart volumetric evaluation after myocardial infarction. *Cardiovasc Ultrasound*. 2020;18:9.

6. Durgin BG, Hahn SA, Schmidt HM, Miller MP, Hafeez N, Mathar I, Freitag D, Sandner P and Straub AC. Loss of smooth muscle CYB5R3 amplifies angiotensin II-induced hypertension by increasing sGC heme oxidation. *JCI Insight*. 2019;4.

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
C57BL/6J	Jackson Laboratories	N/A	M	N/A

Genetically Modified Animals

Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
$Xdh^{fl/fl}Alb\text{-}1^{Cre/Wt\ or\ Wt/Wt}$	Bred at University of Pittsburgh	C57Bl/6J	N/A	N/A
Townes AA and SS	Bred at University of Pittsburgh	C57Bl/6J and 129S	N/A	N/A

Antibodies- Not applicable

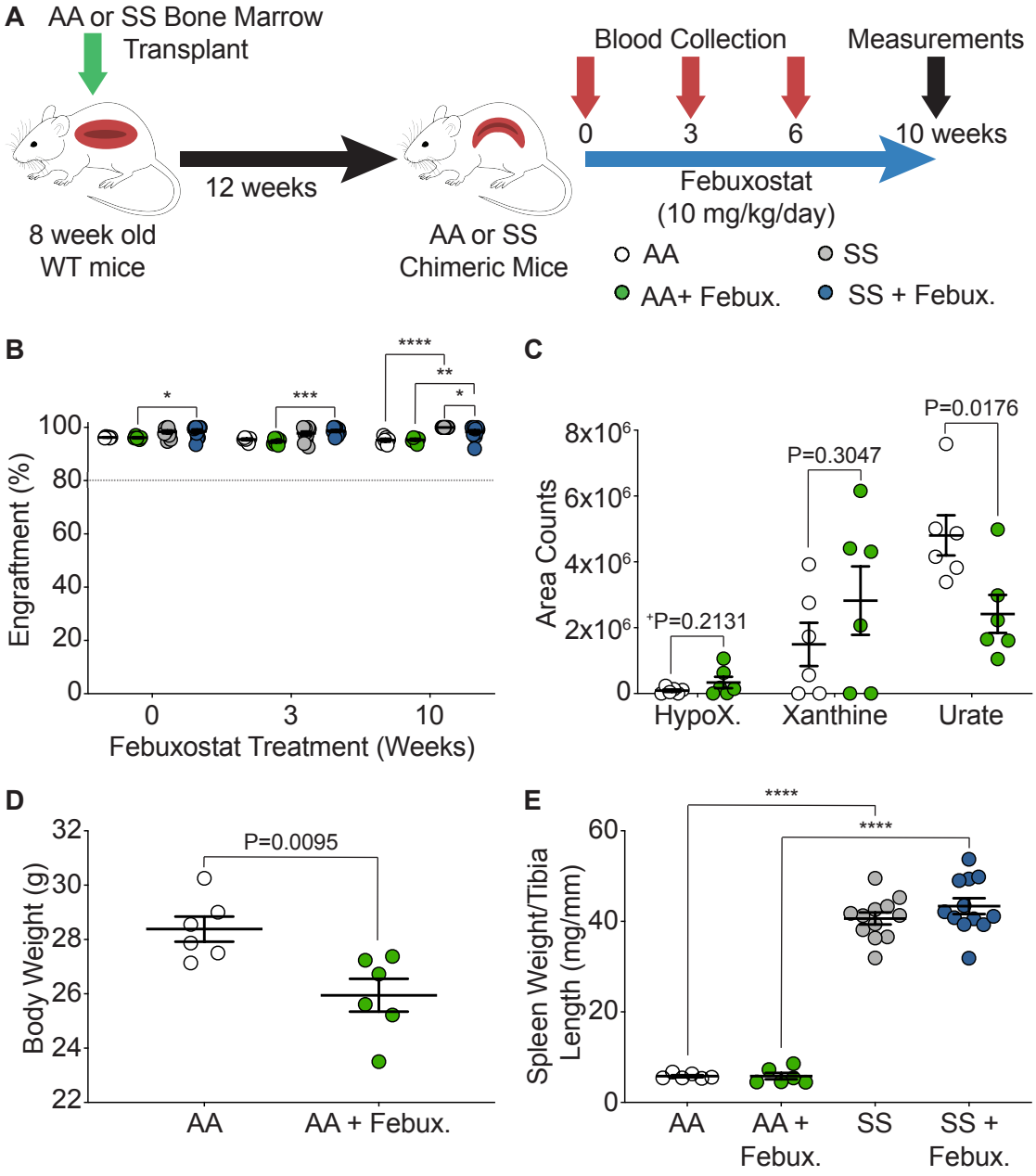
DNA/cDNA Clones- Not applicable

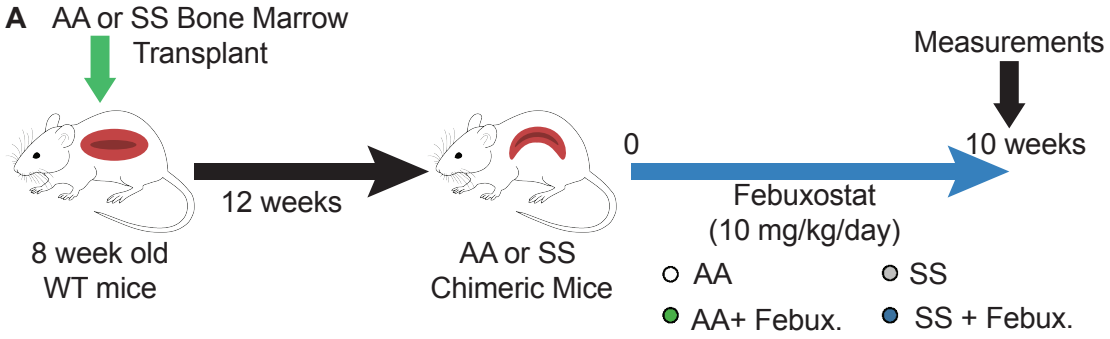
Cultured Cells- Not applicable

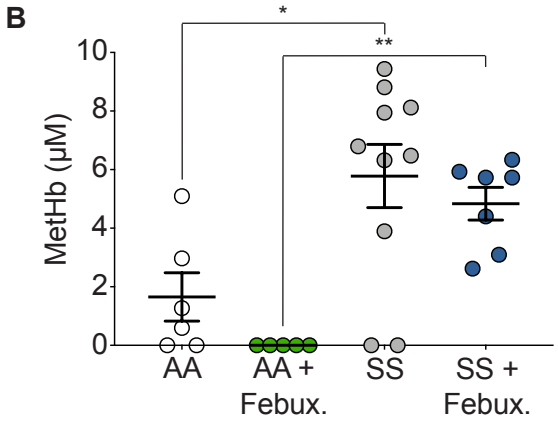
Data & Code Availability- Not applicable

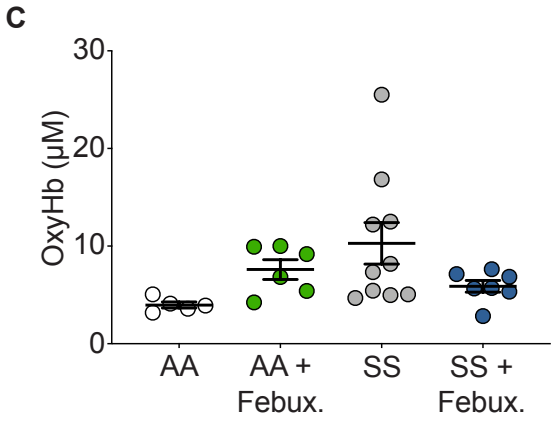
Other- Not applicable

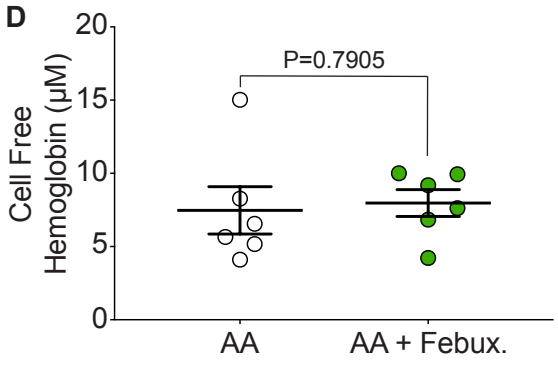
DOI [to be added]

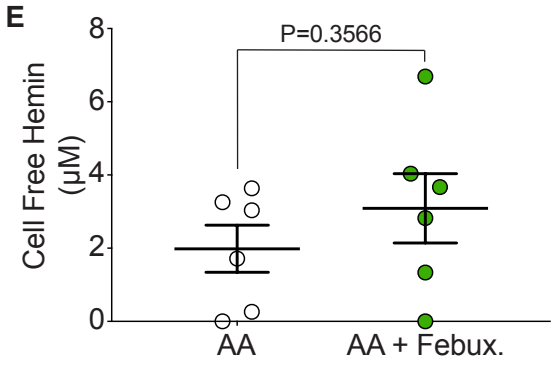

A
AA or SS Bone Marrow Transplant
8 week old WT mice
12 weeks
AA or SS Chimeric Mice
Blood Collection
Measurements
0
3
6
10 weeks
Febuxostat (10 mg/kg/day)
AA
SS
AA+ Febux.
SS + Febux.
B
Engraftment (%)
Febuxostat Treatment (Weeks)
C
Area Counts
P=0.2131
P=0.3047
P=0.0176
HypoX.
Xanthine
Urate
D
Body Weight (g)
P=0.0095
AA
AA + Febux.
E
Spleen Weight/Tibia Length (mg/mm)
AA
AA + Febux.
SS
SS + Febux.

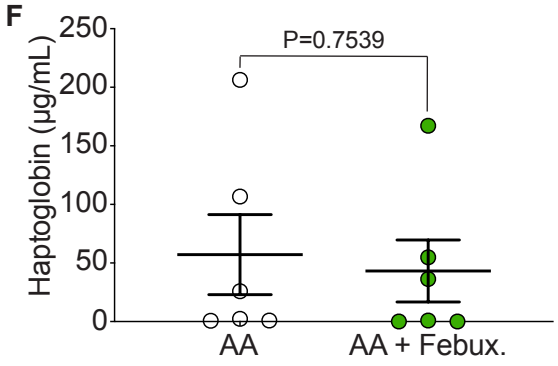

A
AA or SS Bone Marrow Transplant
8 week old WT mice
12 weeks
AA or SS Chimeric Mice
0
Measurements
10 weeks
Febuxostat (10 mg/kg/day)
AA
SS
AA+ Febux.
SS + Febux.

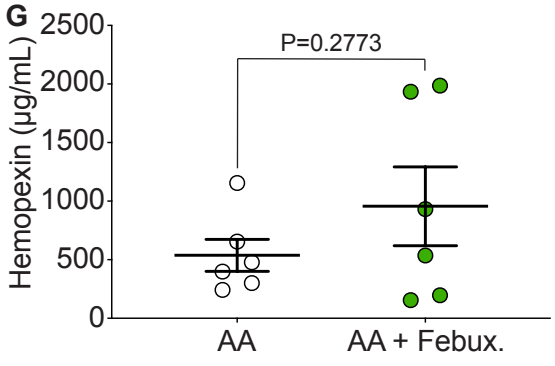

B
MetHb (μM)
*
**
AA
AA + Febux.
SS
SS + Febux.

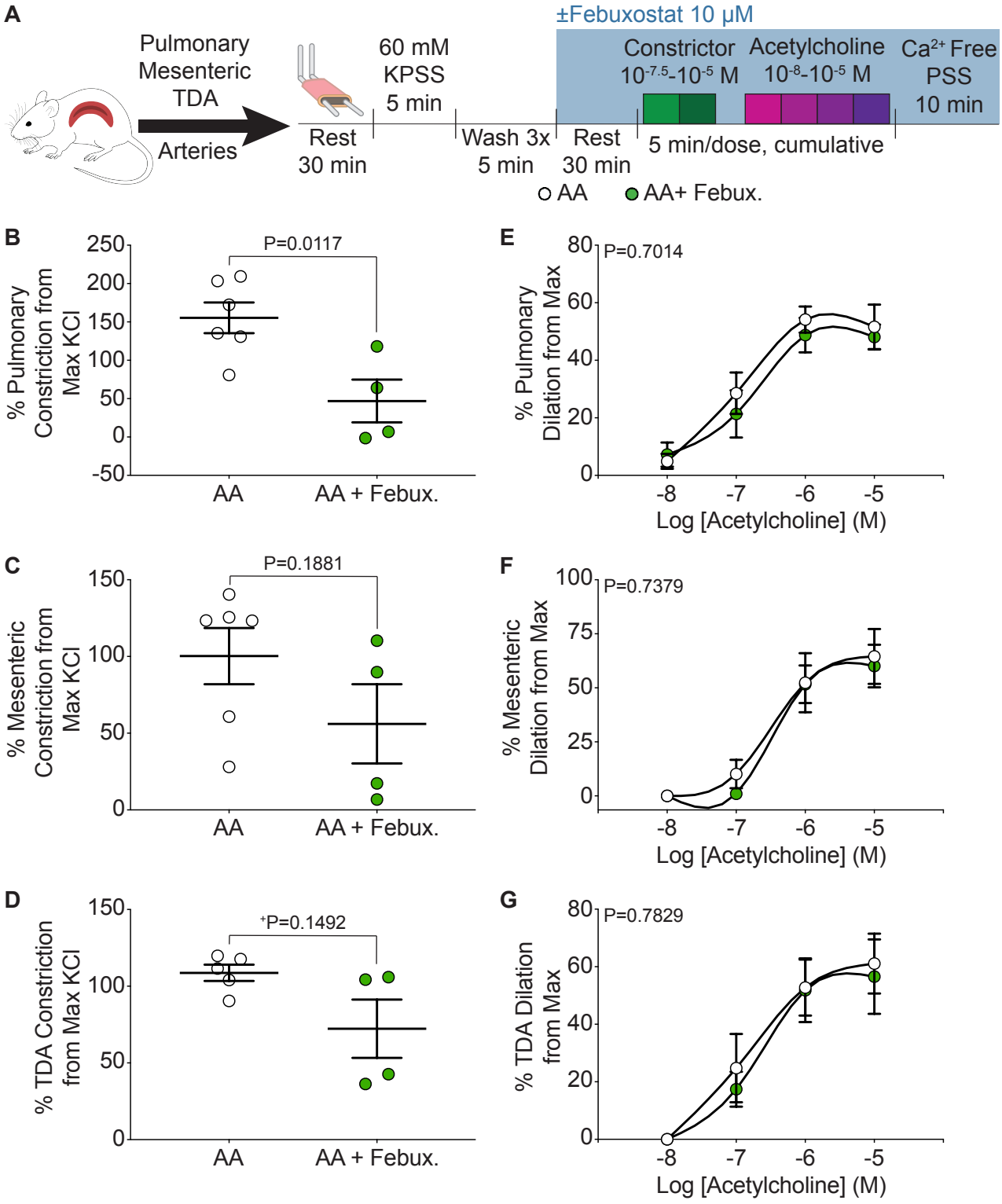

C
OxyHb (μM)
AA
AA + Febux.
SS
SS + Febux.

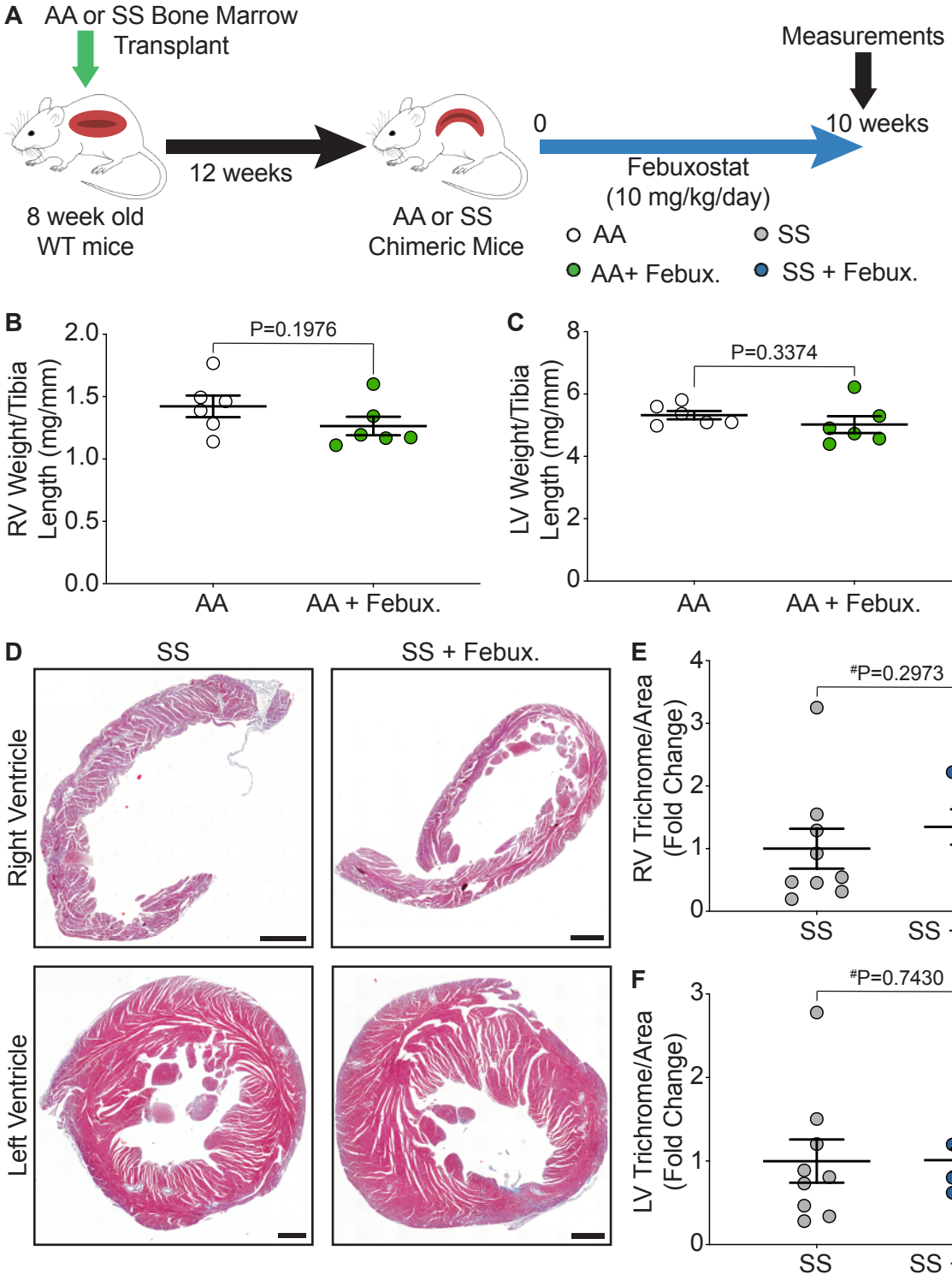

D
Cell Free Hemoglobin (μM)
P=0.7905
AA
AA + Febux.

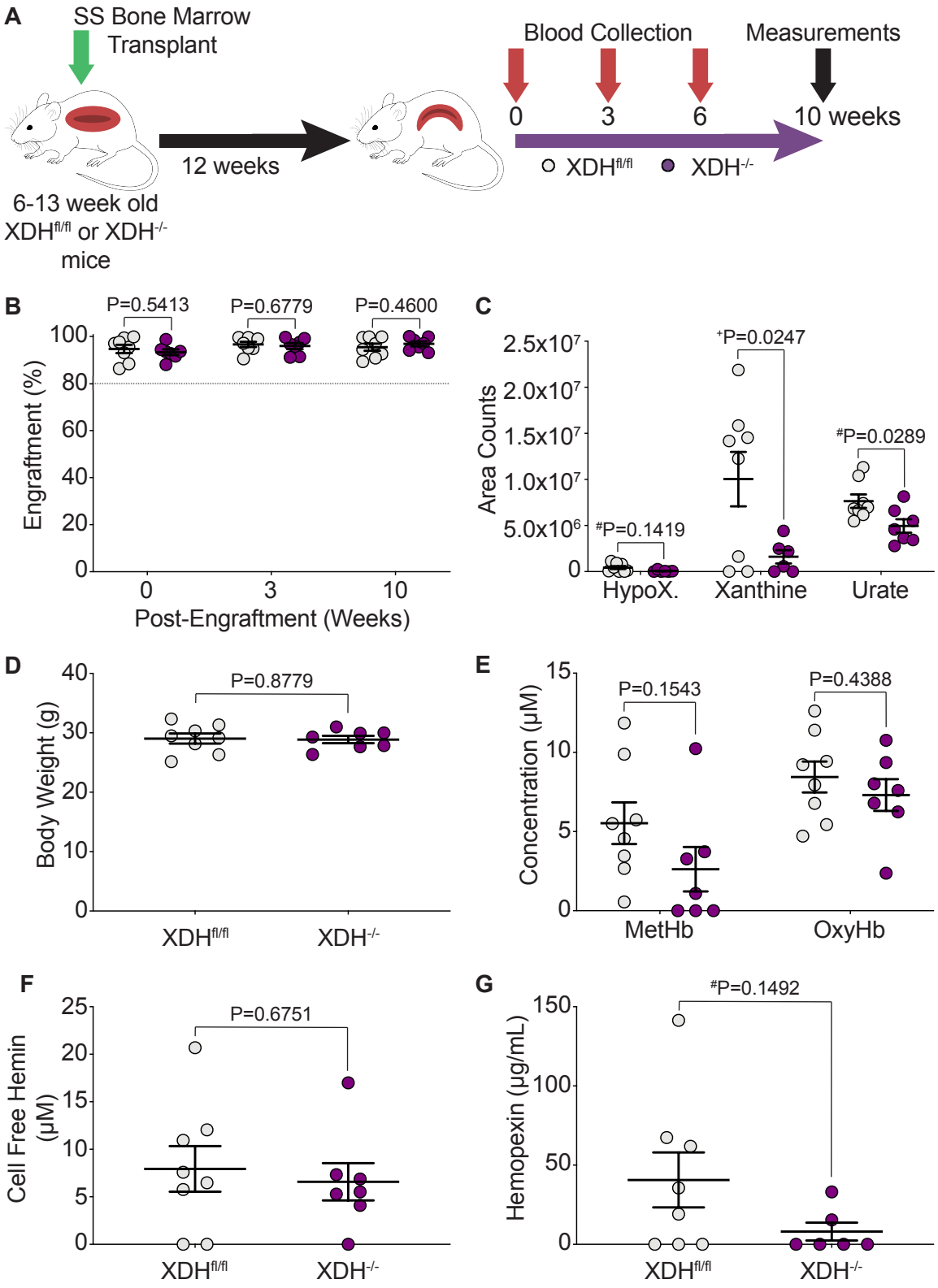

E
Cell Free Hemin (μM)
P=0.3566
AA
AA + Febux.

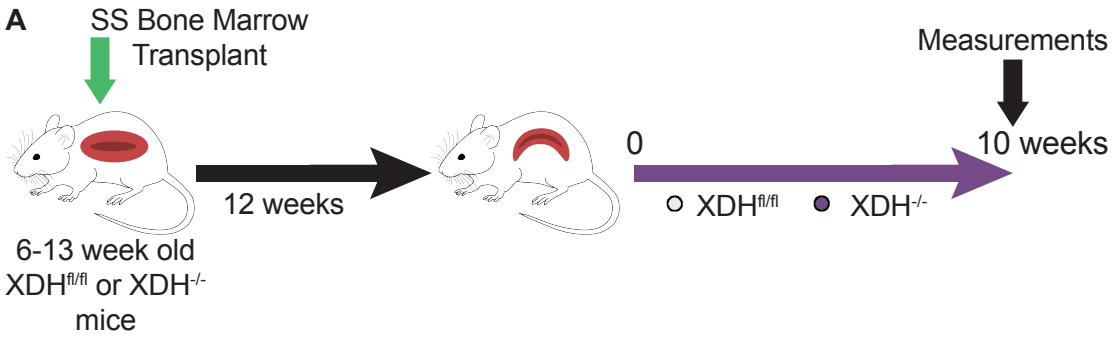

F
Haptoglobin (μg/mL)
P=0.7539
AA
AA + Febux.

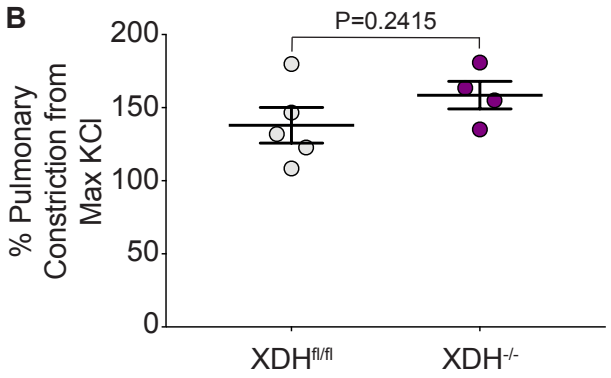

G
Hemopexin (μg/mL)
P=0.2773
AA
AA + Febux.

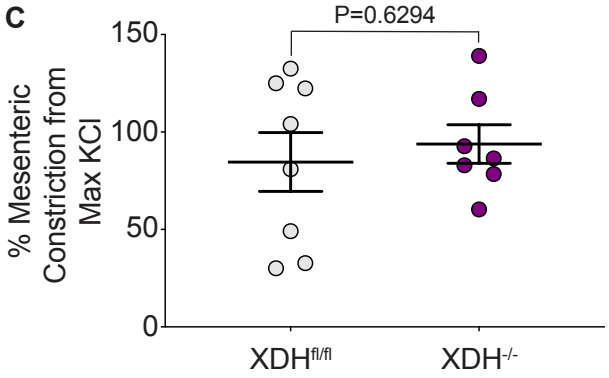

A
Pulmonary
Mesenteric
TDA
Arteries
Rest
30 min
60 mM
KPSS
5 min
Wash 3x
5 min
Rest
30 min
±Febuxostat 10 μM
Constrictor
10-7.5-10-5 M
Acetylcholine
10-8-10-5 M
Ca2+ Free
PSS
10 min
5 min/dose, cumulative
AA
AA+ Febux.
B
% Pulmonary Constriction from Max KCl
P=0.0117
AA
AA + Febux.
C
% Mesenteric Constriction from Max KCl
P=0.1881
AA
AA + Febux.
D
% TDA Constriction from Max KCl
+P=0.1492
AA
AA + Febux.
E
% Pulmonary Dilation from Max
P=0.7014
Log [Acetylcholine] (M)
F
% Mesenteric Dilation from Max
P=0.7379
Log [Acetylcholine] (M)
G
% TDA Dilation from Max
P=0.7829
Log [Acetylcholine] (M)

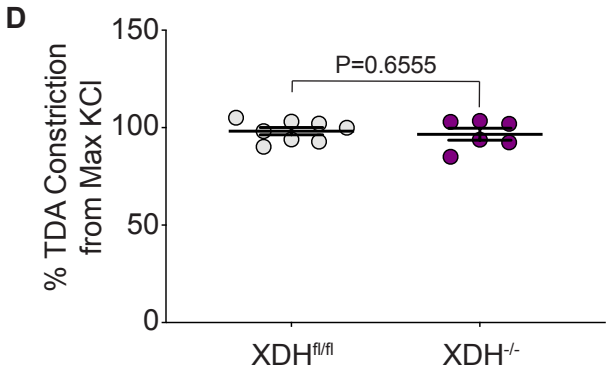

A
AA or SS Bone Marrow Transplant
8 week old WT mice
12 weeks
AA or SS Chimeric Mice
0
Measurements
10 weeks
Febuxostat (10 mg/kg/day)
AA
SS
AA+ Febux.
SS + Febux.
B
RV Weight/Tibia Length (mg/mm)
P=0.1976
AA
AA + Febux.
C
LV Weight/Tibia Length (mg/mm)
P=0.3374
AA
AA + Febux.
D
SS
SS + Febux.
Right Ventricle
Left Ventricle
E
RV Trichrome/Area (Fold Change)
#P=0.2973
SS
SS + Febux.
F
LV Trichrome/Area (Fold Change)
#P=0.7430
SS
SS + Febux.

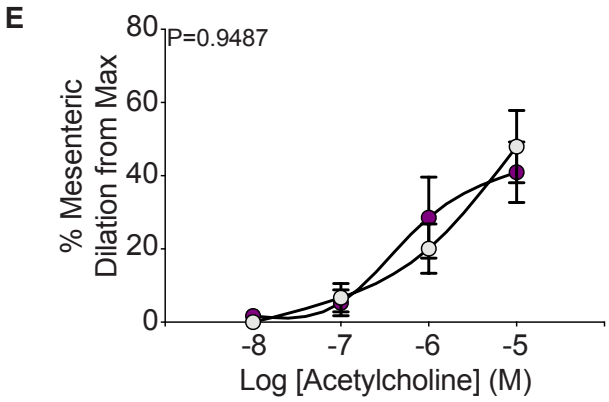

A
SS Bone Marrow Transplant
6-13 week old XDH^fl/fl or XDH^-/- mice
12 weeks
Blood Collection
Measurements
0
3
6
10 weeks
XDH^fl/fl
XDH^-/-
B
P=0.5413
P=0.6779
P=0.4600
Engraftment (%)
Post-Engraftment (Weeks)
C
+P=0.0247
#P=0.0289
#P=0.1419
Area Counts
HypoX.
Xanthine
Urate
D
P=0.8779
Body Weight (g)
E
P=0.1543
P=0.4388
Concentration (µM)
MetHb
OxyHb
F
P=0.6751
Cell Free Hemin (µM)
G
#P=0.1492
Hemopexin (µg/mL)

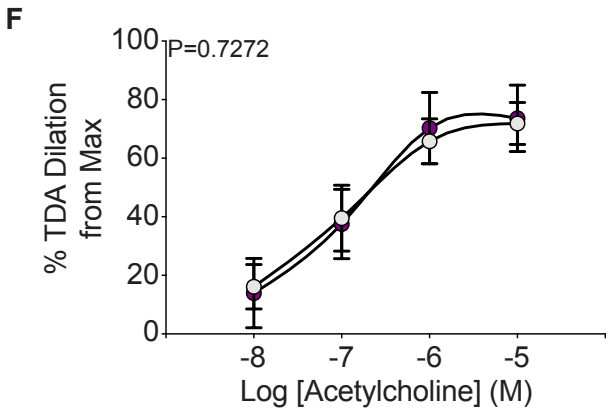

A
SS Bone Marrow Transplant
6-13 week old XDH^fl/fl or XDH^-/- mice
12 weeks
0
10 weeks
Measurements
XDH^fl/fl
XDH^-/-

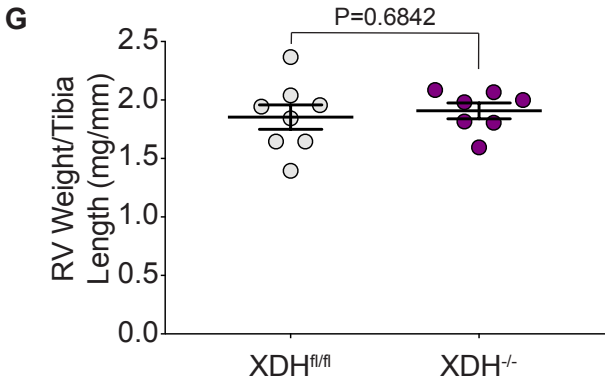

B
P=0.2415
% Pulmonary Constriction from Max KCl
200
150
100
50
0
XDH^fl/fl
XDH^-/-

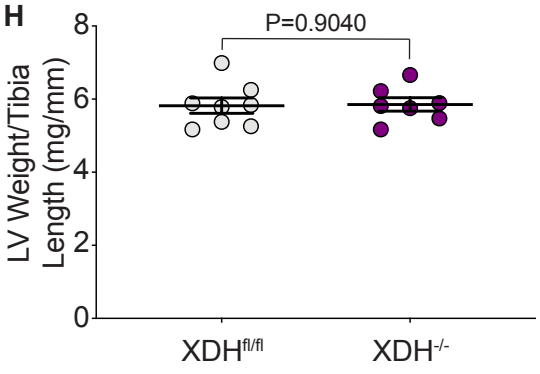

C
P=0.6294
% Mesenteric Constriction from Max KCl
150
100
50
0
XDH^fl/fl
XDH^-/-


D
P=0.6555
% TDA Constriction from Max KCl
150
100
50
0
XDH^fl/fl
XDH^-/-


E
P=0.9487
% Mesenteric Dilation from Max
80
60
40
20
0
-8
-7
-6
-5
Log [Acetylcholine] (M)


F
P=0.7272
% TDA Dilation from Max
100
80
60
40
20
0
-8
-7
-6
-5
Log [Acetylcholine] (M)


G
P=0.6842
RV Weight/Tibia Length (mg/mm)
2.5
2.0
1.5
1.0
0.5
0.0
XDH^fl/fl
XDH^-/-


H
P=0.9040
LV Weight/Tibia Length (mg/mm)
8
6
4
2
0
XDH^fl/fl
XDH^-/-

Supplemental Table I. Febuxostat treatment significantly decreased uric acid concentration and XO activity in plasma and tissues of AA control mice at 10 weeks post-initiation of treatment with effects on plasma detected as early as 3 weeks.

		0 week		3 week		10 week		Significance
		AA *(n=6)*	AA + Febux. *(n=5-6)*	AA *(n=6)*	AA + Febux. *(n=6)*	AA *(n=6)*	AA + Febux. *(n=6)*	AA 0 vs AA + Febux. 0 **AA 3 vs AA + Febux. 3** AA 10 vs. AA + Febux. 10
Plasma	Uric Acid, µM	0.62 ± 0.09	1.05 ± 0.07	1.52 ± 0.15	0.13 ± 0.05	0.95 ± 0.10	0.28 ± 0.06	P=0.011, **P<0.001** P=0.001
	XO Activity, µUnits/mL	58.00 ± 8.81	64.34 ± 5.05	90.95 ± 8.92	13.05 ± 5.96	57.06 ± 6.13	16.67 ± 3.46	P=0.894, **P<0.001** P=0.001
Liver	Uric Acid, µM	-	-	-	-	56.43 ± 3.87	9.97 ± 2.89	#P=0.002
	XO Activity, mUnits/mg	-	-	-	-	15.86 ± 0.92	3.04 ± 0.93	P<0.001
Lung	Uric Acid, µM	-	-	-	-	13.78 ± 0.78	8.07 ± 1.69	P=0.012
	XO Activity, mUnits/mg	-	-	-	-	28.62 ± 1.24	16.82 ± 3.46	#P=0004
Kidney	Uric Acid, µM	-	-	-	-	6.60 ± 0.32	0.44 ± 0.17	P<0.001
	XO Activity, mUnits/mg	-	-	-	-	2.13 ± 0.08	0.14 ± 0.05	P<0.001

Definition of abbreviations: XO = xanthine oxidase; febux = febuxostat; - = measurement not made. Values are mean ± SEM using a 2-way ANOVA with Sidak's multiple comparison test for plasma measurements. Values are mean ± SEM using an unpaired t test for tissue measurements unless otherwise noted. #Values are mean ± SEM using a Mann-Whitney test. P values are in normal font for AA 0 week vs. AA + febuxostat 0 week, **bolded** for AA 3 week vs. AA + febuxostat 3 week, and underlined for AA 10 week vs. AA + febuxostat 10 week.

Supplemental Table II. Febuxostat treatment significantly decreased uric acid concentration and XO activity in plasma and tissues of SS sickle mice at 10 weeks post-initiation of treatment with effects on plasma detected as early as 3 weeks.

		0 week		3 week		10 week		Significance
		SS *(n=12)*	SS + Febux. *(n=12)*	SS *(n=11-12)*	SS + Febux. *(n=11)*	SS *(n=12)*	SS + Febux. *(n=12)*	SS 0 vs SS + Febux. 0 **SS 3 vs SS + Febux. 3** SS 10 vs. SS + Febux. 10
Plasma	Uric Acid, µM	1.32 ± 0.14	1.099 ± 0.29	0.55 ± 0.13	0.23 ± 0.07	2.65 ± 0.15	0.94 ± 0.13	P=0.8814, **P=0.1189** P<0.0001
	XO Activity, µUnits/mL	79.08 ± 8.52	65.95 ± 17.44	45.62 ± 9.72	13.56 ± 4.30	159.1 ± 8.78	52.20 ± 9.74	P=0.8814, **P=0.0279** P=<0.0001
Liver	Uric Acid, µM	-	-	-	-	85.18 ± 2.04	2.97 ± 0.51	P<0.0001
	XO Activity, mUnits/mg	-	-	-	-	29.40 ± 1.00	0.97 ± 0.17	P<0.0001
Lung	Uric Acid, µM	-	-	-	-	11.50 ± 0.60	4.34 ± 0.54	P<0.0001
	XO Activity, mUnits/mg	-	-	-	-	12.60 ± 0.94	4.80 ± 0.62	+P<0.0001
Kidney	Uric Acid, µM	-	-	-	-	10.88 ± 0.63	0.29 ± 0.06	#P<0.0001
	XO Activity, mUnits/mg	-	-	-	-	2.78 ± 0.20	0.08 ± 0.01	+P<0.0001

Definition of abbreviations: XO = xanthine oxidase; febux = febuxostat; - = measurement not made. Values are mean ± SEM using a 2-way ANOVA with Sidak's multiple comparison test for plasma measurements. Values are mean ± SEM using an unpaired t test for tissue measurements unless otherwise noted. #Values are mean ± SEM using a Mann-Whitney test. +Values are mean ± SEM using an unpaired t test with Welch's correction. P values are in normal font for SS 0 week vs. febuxostat 0 week, **bolded** for SS 3 week vs. febuxostat 3 week, and underlined for SS 10 week vs. febuxostat 10 week.

Supplemental Table III. Blood cell indices for Townes AA bone marrow transplanted mice at 0, 3, 6, and 10 weeks of drinking normal water (AA) or febuxostat treated water (AA + Febux).

	0 week		3 week		6 week		10 week		Significance
	AA *(n=6)*	AA + Febux. *(n=5-6)*	AA *(n=5-6)*	AA + Febux. *(n=4-6)*	AA *(n=6)*	AA + Febux. *(n=5)*	AA *(n=6)*	AA + Febux. *(n=6)*	AA 0 vs AA + Febux. 0 **AA 3 vs AA + Febux. 3** *AA 6 vs AA + Febux. 6* AA 10 vs. AA + Febux. 10
WBC, 10^3/µL	15.33 ± 0.69	14.28 ± 0.16	19.67 ± 0.72	15.53 ± 1.06	15.97 ± 0.65	12.26 ± 1.30	18.42 ± 1.40	16.57 ± 1.17	P=0.918, **P=0.017** *P=0.052,* P=0.551
RBC, 10^6/µL	10.85 ± 0.26	10.88 ± 0.22	10.96 ± 0.08	11.03 ± 0.22	10.51 ± 0.17	11.07 ± 0.30	10.78 ± 0.20	11.15 ± 0.29	P>0.999, **P=0.999** *P=0.323,* P=0.674
HCT, %	35.00 ± 0.83	35.08 ± 0.62	34.88 ± 0.18	35.17 ± 0.64	33.38 ± 0.51	35.30 ± 0.79	34.13 ± 0.67	35.63 ± 1.07	P>0.999, **P=0.997** *P=0.254,* P=0.441
MCV, fl	32.35 ± 0.23	32.20 ± 0.09	31.83 ± 0.19	31.87 ± 0.10	31.78 ± 0.13	31.88 ± 0.16	31.65 ± 0.16	31.92 ± 0.20	P=0.999, **P>0.999** *P=0.991,* P=0.694
RDW, %	27.35 ± 0.20	27.30 ± 0.10	27.20 ± 0.03	27.28 ± 0.08	27.27 ± 0.24	28.34 ± 0.41	27.27 ± 0.14	28.52 ± 0.32	P>0.999, **P=0.998** *P=0.006,* P=0.001
HGB, g/dL	12.15 ± 0.25	12.37 ± 0.20	12.38 ± 0.05	12.30 ± 0.23	11.93 ± 0.13	12.62 ± 0.31	12.18 ± 0.22	12.67 ± 0.28	P=0.927, **P=0.998** *P=0.142,* P=0.400
MCH, pg	11.20 ± 0.10	11.40 ± 0.07	11.30 ± 0.05	11.13 ± 0.09	11.42 ± 0.10	11.42 ± 0.05	11.32 ± 0.05	11.35 ± 0.06	P=0.221, **P=0.388** *P>0.999,* P=0.996
PLT, 10^3/µL	762.50 ± 19.80	743.83 ± 16.83	770.33 ± 17.72	689.67 ± 33.45	718.17 ± 39.24	810.00 ± 24.03	850.67 ± 16.95	924.33 ± 46.91	P=0.985, **P=0.205** *P=0.146,* P=0.282
MPV, fl	6.57 ± 0.03	6.60 ± 0.05	6.58 ± 0.08	6.60 ± 0.00	6.65 ± 0.07	6.72 ± 0.06	6.43 ± 0.06	6.55 ± 0.02	P=0.986, **P=0.999** *P=0.847,* P=0.414
Retic, %	5.93 ± 0.40	5.28 ± 0.12	3.98 ± 0.18	3.65 ± 0.22	4.95 ± 0.21	4.94 ± 0.30	4.88 ± 0.22	5.32 ± 0.27	P=0.259, **P=0.821** *P>0.999,* P=0.640

Definition of abbreviations: febux = febuxostat; WBC = white blood cells; RBC = red blood cells; HCT = hematocrit; MCV = mean corpuscular volume; RDW = red blood cell distribution width; HGB = hemoglobin; MCH = mean corpuscular hemoglobin; PLT = platelets; MPV = mean platelet volume; retic = reticulocytes. Values are mean ± SEM using a 2 way ANOVA with Sidak's multiple comparison test. P values are in normal font for AA 0 week vs. AA + febuxostat 0 week, **bolded** for AA 3 week vs. AA + febuxostat 3 week, *italicized* for AA 6 week vs. AA + febuxostat 6 week, and underlined for AA 10 week vs. AA + febuxostat 10 week.

Supplemental Table IV. Blood cell indices for Townes SS bone marrow transplanted mice at 0, 3, 6, and 10 weeks of drinking normal water (SS) or febuxostat treated water (SS + Febux).

	0 week		3 week		6 week		10 week		Significance
	SS *(n=12)*	SS + Febux. *(n=12)*	SS *(n=11-12)*	SS + Febux. *(n=10-12)*	SS *(n=12)*	SS + Febux. *(n=12)*	SS *(n=11-12)*	SS + Febux. *(n=12)*	SS 0 vs SS + Febux. 0 **SS 3 vs SS + Febux. 3** *SS 6 vs SS + Febux. 6* SS 10 vs. SS + Febux. 10
WBC, 10^3/µL	16.74 ± 1.20	19.32 ± 1.35	23.72 ± 1.51	21.47 ± 0.94	26.14 ± 0.86	23.73 ± 1.11	26.60 ± 1.43	25.48 ± 1.09	P=0.428, **P=0.622,** *P=0.494* , P=0.942
RBC, 10^6/µL	6.09 ± 0.16	5.94 ± 0.14	6.52 ± 0.32	6.41 ± 0.08	6.17 ± 0.19	6.61 ± 0.11	6.42 ± 0.17	6.94 ± 0.14	P=0.966, **P=0.986,** *P=0.290* , P=0.151
HCT, %	27.58 ± 0.83	26.67 ± 0.68	27.80 ± 0.79	28.53 ± 0.36	26.44 ± 0.73	28.28 ± 0.56	28.05 ± 0.35	30.64 ± 0.66	P=0.776, **P=0.897,** *P=0.165* , P=0.024
MCV, fl	44.95 ± 0.33	44.73 ± 0.26	44.49 ± 0.35	44.51 ± 0.26	42.92 ± 0.32	42.75 ± 0.37	44.59 ± 0.42	44.13 ± 0.29	P=0.983, **P>0.999,** *P=0.994* , P=0.780
RDW, %	35.00 ± 0.40	35.01 ± 0.23	35.57 ± 0.19	36.41 ± 0.15	36.91 ± 0.26	36.81 ± 0.26	35.93 ± 0.39	36.93 ± 0.29	P>0.999, **P=0.147,** *P=0.999* , P=0.057
HGB, g/dL	9.72 ± 0.25	9.44 ± 0.23	9.82 ± 0.24	10.04 ± 0.17	9.63 ± 0.25	10.18 ± 0.19	9.77 ± 0.10	10.67 ± 0.23	P=0.831, **P=0.910,** *P=0.234* , P=0.017
MCH, pg	15.97 ± 0.09	15.93 ± 0.09	15.76 ± 0.07	15.68 ± 0.11	15.63 ± 0.14	15.43 ± 0.10	15.55 ± 0.20	15.37 ± 0.08	P=0.999, **P=0.983,** *P=0.648* , P=0.717
PLT, 10^3/µL	421.00 ± 14.79	417.33 ± 12.69	445.50 ± 31.24	470.10 ± 7.94	535.58 ± 21.88	550.83 ± 18.12	513.50 ± 18.84	573.58 ± 19.45	P=0.999, **P=0.866,** *P=0.969* , P=0.119
MPV, fl	6.37 ± 0.04	6.39 ± 0.04	6.43 ± 0.06	6.58 ± 0.04	6.48 ± 0.03	6.62 ± 0.05	6.47 ± 0.07	6.56 ± 0.05	P=0.993, **P=0.130,** *P=0.143* , P=0.539
Retic, %	44.7 ± 0.99	44.74 ± 0.57	-	-	44.30 ± 0.98	43.08 ± 0.81	46.58 ± 1.16	44.16 ± 1.22	P=0.984, *P=0.764* , P=0.234

Definition of abbreviations: febux = febuxostat; WBC = white blood cells; RBC = red blood cells; HCT = hematocrit; MCV = mean corpuscular volume; RDW = red blood cell distribution width; HGB = hemoglobin; MCH = mean corpuscular hemoglobin; PLT = platelets; MPV = mean platelet volume; retic = reticulocytes; - = measurement not made. Values are mean ± SEM using a 2 way ANOVA with Sidak's multiple comparison test. P values are in normal font for SS 0 week vs. SS + febuxostat 0 week, **bolded** for SS 3 week vs. SS + febuxostat 3 week, *italicized* for SS 6 week vs. SS + febuxostat 6 week, and underlined for SS 10 week vs. SS + febuxostat 10 week.

Supplemental Table V. Closed chest right heart catheterization indices for Townes AA, AA + febuxostat, SS, SS + febuxostat, *Xdh* fl/fl, and H*Xdh* -/- chimeras ten weeks post-engraftment.

	AA *(n=6)*	AA + Febux. *(n=6)*	SS *(n=9-10)*	SS + Febux. *(n=10)*	$XDH^{fl/fl}$ *(n=8)*	$XDH^{-/-}$ *(n=7)*	Significance AA vs AA + Febux **SS vs SS + Febux.** *AA vs SS* AA + Febux. vs. SS + Febux.	Significance $XDH^{fl/fl}$ vs. $XDH^{-/-}$
RV Max Pressure mmHg	28.21 ± 1.15	29.19 ± 1.40	30.29 ± 1.52	32.50 ± 0.79	32.53 ± 0.58	33.65 ± 0.87	P=0.983, **P=0.550,** *P=0.720*, P=0.302	P=0.294
Heart Rate bpm	581.38 ± 32.11	592.70 ± 21.25	620.69 ± 11.84	608.46 ± 12.03	610.11 ± 18.97	639.46 ± 11.39	P=0.992, **P=0.972,** *P=0.451*, P=0.958	P=0.224
RV End Diastolic Pressure mmHg	3.07 ± 0.37	3.13 ± 0.20	2.28 ± 0.25	2.63 ± 0.17	2.39 ± 0.23	2.95 ± 0.21	P>0.999, **P=0.720,** *P=0.139*, P=0.532	P=0.096
RV Max dP/dt	1716.33 ± 163.67	1910.50 ± 181.50	2309.80 ± 138.69	2546.20 ± 102.23	2395.38 ± 100.78	2564 ± 117.02	P=0.876, **P=0.583,** *P=0.030*, P=0.018	P=0.291
RV Min dP/dt	-1670.50 ± 141.90	-1777.83 ± 156.67	-1703.00 ± 138.30	-1890.80 ± 79.29	-1940.63 ± 86.39	-2035.71 ± 112.33	P=0.977, **P=0.689,** *P>0.999*, P=0.958	P=0.508
RV Contractile Index 1/s	60.33 ± 3.67	64.80 ± 3.01	75.99 ± 1.73	78.13 ± 1.40	73.53 ± 2.38	75.98 ± 1.73	P=0.651, **P=0.912,** *P<0.001*, P=0.001	P=0.431
PA Mean Pressure mmHg	18.89 ± 0.74	19.52 ± 0.91	20.24 ± 0.99	21.67 ± 0.51	21.70 ± 0.38	22.42 ± 0.56	P=0.983, **P=0.550,** *P=0.720*, P=0.302	P=0.294
Tau (W) ms	5.45 ± 0.17	6.20 ± 0.40	5.79 ± 0.38	6.27 ± 0.86	6.13 ± 0.55	5.82 ± 0.32	P=0.917, **P=0.960,** *P=0.994*, P>0.999	P=0.650

Definition of abbreviations: febux = febuxostat; RV = right ventricle; PA = pulmonary artery. Values are mean ± SEM using a 1 way ANOVA with Sidak's multiple comparison test for WT AA and SS mice. P values are in normal font for AA vs. AA + febuxostat, **bolded** for SS vs. SS + febuxostat, *italicized* for AA vs. SS, and underlined for AA + febuxostat vs. SS + febuxostat 10. Values are mean ± SEM using an unpaired t test for *Xdh* mice.

Supplemental Table VI. Right ventricle echocardiogram indices for Townes AA, AA + febuxostat, SS, SS + febuxostat, *Xdh* fl/fl, and H*Xdh* -/- chimeras ten weeks post-engraftment.

	Right Ventricle						Significance	Significance
	AA *(n=5-6)*	AA + Febux. *(n=5-6)*	SS *(n=8-11)*	SS + Febux. *(n=7-11)*	$XDH^{fl/fl}$ *(n=5-8)*	$XDH^{-/-}$ *(n=3-7)*	AA vs AA + Febux **SS vs SS + Febux.** *AA vs SS* AA + Febux. vs. SS + Febux.	$XDH^{fl/fl}$ vs. $XDH^{-/-}$
TV A mm/s	334.05 ± 24.30	339.16 ± 14.01	327.43 ± 19.15	320.51 ± 28.81	325.22 ± 76.76	362.41 ± 22.69	P>0.999, **P=0.999,** *P>0.999* , P=0.981	#P=0.656
TV E ms	192.97 ± 3.82	198.63 ± 25.32	439.54 ± 25.44	360.25 ± 32.00	330.07 ± 39.41	375.28 ± 72.33	P>0.999, **P=0.126,** *P<0.001* , P=0.004	P=0.567
TV LW A' mm/s	-36.36 ± 4.03	-32.82 ± 3.71	-33.01 ± 3.19	-40.73 ± 3.10	-30.73 ± 5.66	-33.06 ± 2.77	P=0.949, **P=0.343,** *P=0.940* , P=0.435	P=0.744
TV LW E' ms	-28.17 ± 3.36	-29.27 ± 2.57	-28.84 ± 2.78	-38.58 ± 3.23	-25.15 ± 5.33	-27.65 ± 6.25	P=0.999, **P=0.088,** *P>0.999* , P=0.186	P=0.768
TV E/A ms	0.55 ± 0.03	0.58 ± 0.06	1.40 ± 0.12	1.22 ± 0.16	1.24 ± 0.22	1.02 ± 0.17	P>0.999, **P=0.711,** *P=0.001* , P=0.020	P=0.469
TV E/e' mm/s	-8.53 ± 0.96	-7.02 ± 1.06	-16.58 ± 2.98	-7.85 ± 1.61	-15.11 ± 2.79	-10.32 ± 1.85	P=0.988, **P=0.025,** *P=0.073* , P=0.998	P=0.272
PAT mm/s	22.59 ± 0.78	20.51 ± 0.99	20.96 ± 0.80	20.73 ± 0.57	20.60 ± 1.59	22.62 ± 1.33	P=0.405, **P=0.999,** *P=0.516* , P>0.999	P=0.349
PET	58.98 ± 1.64	57.04 ± 1.04	63.23 ± 1.44	60.48 ± 2.23	66.88 ± 3.23	70.67 ± 2.70	P=0.956, **P=0.675,** *P=0.438* , P=0.634	P=0.385
PA Peak Velocity	-529.39 ± 26.32	-571.45 ± 29.89	-599.00 ± 18.97	-573.34 ± 24.52	-458.73 ± 35.32	-470.89 ± 23.80	P=0.782, **P=0.876,** *P=0.234* , P>0.999	P=0.780
PAT/PET	0.39 ± 0.02	0.36 ± 0.01	0.33 ± 0.02	0.34 ± 0.01	0.31 ± 0.02	0.32 ± 0.01	P=0.845, **P=0.962,** *P=0.202* , P=0.984	P=0.601
FAC %	50.76 ± 2.80	43.99 ± 3.87	39.41 ± 1.31	46.85 ± 3.45	34.82 ± 0.67	46.30 ± 4.25	P=0.546, **P=0.184,** *P=0.053* , P=0.944	*P=0.036
RVID mm	1.38 ± 0.04	1.38 ± 0.15	1.68 ± 0.06	1.60 ± 0.10	1.80 ± 0.08	1.88 ± 0.06	P>0.999., **P=0.931,** *P=0.139* , P=0.385	P=0.4618
RVFW mm	0.40 ± 0.02	0.38 ± 0.01	0.40 ± 0.02	0.34 ± 0.01	0.39 ± 0.02	0.43 ± 0.03	P=0.987, **P=0.115,** *P=0.999* , P=0.622	P=0.305
TAPSE mm	1.15 ± 0.07	1.14 ± 0.09	1.24 ± 0.07	1.36 ± 0.09	1.07 ± 0.10	1.22 ± 0.07	P>0.999, **P=0.737,** *P=0.925* , P=0.320	P=0.266

Definition of abbreviations: febux = febuxostat; TV = tricuspid valve; A = peak velocity of late transmitral flow; E = peak velocity of early diastolic transmitral flow; LW = left wall; A' = peak velocity of diastolic mitral annular motion; E' = peak velocity of early diastolic mitral annular motion; PAT = pulmonary acceleration time; PET = pulmonary ejection time; PA = pulmonary artery; FAC = fractional area change; RVID = right ventricular internal diameter; RVFW = right ventricular free wall; TAPSE = tricuspid annular plane systolic excursion. Values are mean ± SEM using a 1 way ANOVA for WT AA and SS mice. P values are in normal font for AA vs. AA + febuxostat, **bolded** for SS vs. SS + febuxostat, *italicized* for AA vs. SS, and underlined for AA + febuxostat vs. SS + febuxostat 10. Values are mean ± SEM using an unpaired t test for XDH mice unless otherwise noted. #Values are mean ± SEM using a Mann-Whitney test.

Supplemental Table VII. Left ventricle echocardiogram indices for Townes AA, AA + febuxostat, SS, SS + febuxostat, *Xdh* fl/fl, and H*Xdh* -/- chimeras ten weeks post-engraftment.

	Left Ventricle						Significance	Significance
	AA *(n=5-6)*	AA + Febux. *(n=6)*	SS *(n=10-11)*	SS + Febux. *(n=9-11)*	$XDH^{fl/fl}$ *(n=6-8)*	$XDH^{-/-}$ *(n=7)*	AA vs AA + Febux **SS vs SS + Febux.** *AA vs SS* AA + Febux. vs. SS + Febux.	$XDH^{fl/fl}$ vs. $XDH^{-/-}$
A' mm/s	-22.02 ± 2.16	-21.48 ± 2.07	-27.77 ± 2.34	-23.76 ± 1.31	-23.03 ± 2.08	-22.40 ± 2.32	P>0.999, **P=0.437**, *P=0.241* , P=0.916	P=0.843
AET ms	56.53 ± 0.92	55.28 ± 1.37	57.83 ± 1.61	55.40 ± 1.55	56.01 ± 2.21	56.56 ± 3.59	P=0.983, **P=0.637**, *P=0.969* , P>0.999	P=0.894
E' mm/s	-29.30 ± 2.43	-32.22 ± 2.41	-32.06 ± 1.64	-33.60 ± 2.25	-30.41 ± 2.07	-28.84 ± 3.56	P=0.887, **P=0.967**, *P=0.857* , P=0.988	P=0.710
IVCT ms	14.77 ± 0.88	15.97 ± 0.88	13.16 ± 0.88	12.31 ± 0.70	13.78 ± 2.10	12.91 ± 1.52	P=0.876, **P=0.894**, *P=0.610* , P=0.027	P=0.748
IVRT ms	15.51 ± 1.11	15.51 ± 1.74	15.13 ± 0.62	15.68 ± 1.86	17.55 ± 0.13	15.03 ± 1.53	P>0.999, **P=0.997**, *P>0.999* , P>0.999	+P=0.151
MV A mm/s	463.06 ± 58.69	378.48 ± 28.71	451.34 ± 17.25	437.95 ± 31.43	398.37 ± 40.40	457.05 ± 58.75	P=0.414, **P=0.996**, *P=0.997* , P=0.638	P=0.416
MV E mm/s	721.20 ± 38.57	723.46 ± 58.07	760.50 ± 24.40	777.47 ± 39.33	598.22 ± 46.68	694.53 ± 39.95	P>0.999, **P=0.994**, *P=0.936* , P=0.825	P=0.147
LV MPI IV	0.54 ± 0.03	0.57 ± 0.04	0.49 ± 0.02	0.47 ± 0.03	0.58 ± 0.06	0.49 ± 0.04	P=0.926, **P=0.971**, *P=0.762* , P=0.110	P=0.222
MV E/A	1.66 ± 0.17	1.94 ± 0.16	1.71 ± 0.07	1.67 ± 0.09	1.61 ± 0.21	1.65 ± 0.20	P=0.458, **P=0.999**, *P=0.998* , P=0.393	P=0.899
MV E/E'	-25.03 ± 1.41	-23.00 ± 2.41	-24.27 ± 1.25	-23.65 ± 1.68	-21.69 ± 1.96	-25.35 ± 1.88	P=0.920, **P=0.997**, *P=0.996* , P=0.998	P=0.203
Heart Rate bpm	490.02 ± 15.76	472.49 ± 22.76	459.62 ± 10.51	465.10 ± 10.83	441.43 ± 16.43	447.17 ± 11.10	P=0.911, **P=0.996**, *P=0.465* , P=0.994	P=0.7831
Diameter;s mm	2.78 ± 0.09	2.64 ± 0.14	2.75 ± 0.12	2.61 ± 0.09	2.75 ± 0.18	2.92 ± 0.17	P=0.911, **P=0.782**, *P>0.999* , P>0.999	P=0.519
Diameter;d mm	3.90 ± 0.11	3.77 ± 0.14	4.14 ± 0.11	4.06 ± 0.10	4.05 ± 0.17	4.11 ± 0.14	P=0.937, **P=0.963**, *P=0.507* , P=0.332	P=0.765
Volume;s µL	29.35 ± 2.51	26.17 ± 3.27	29.24 ± 2.93	25.34 ± 2.03	29.69 ± 4.79	33.82 ± 5.13	P=0.934, **P=0.700**, *P>0.999* , P=0.999	P=0.566
Volume;d µL	66.34 ± 4.60	61.48 ± 5.58	76.66 ± 4.56	73.03 ± 4.00	73.28 ± 7.28	75.75 ± 6.34	P=0.956, **P=0.955**, *P=0.473* , P=0.363	P=0.804
Stroke Volume µL	36.99 ± 2.38	35.32 ± 2.79	47.41 ± 3.15	47.69 ± 3.29	43.59 ± 3.30	41.93 ± 3.42	P=0.997, **P>0.999**, *P=0.144* , P=0.059	P=0.733
Ejection Fraction %	55.87 ± 1.29	58.06 ± 2.30	62.28 ± 2.64	65.17 ± 2.43	60.90 ± 3.03	56.09 ± 3.83	P=0.977, **P=0.837**, *P=0.334* , P=0.241	P=0.337
Fractional Shortening %	28.70 ± 0.85	30.20 ± 1.50	33.64 ± 1.97	35.62 ± 1.75	32.49 ± 2.13	29.30 ± 2.64	P=0.981, **P=0.861**, *P=0.276* , P=0.200	P=0.359
Cardiac Output mL/min	18.08 ± 1.13	16.44 ± 0.75	20.50 ± 0.83	22.06 ± 1.43	19.09 ± 1.32	18.81 ± 1.73	P=0.881, **P=0.772**, *P=0.553* , P=0.012	P=0.900
LV Mass mg	116.73 ± 1.33	115.37 ± 7.12	143.48 ± 5.52	159.86 ± 9.27	130.30 ± 10.74	136.08 ± 12.10	P>0.999., **P=0.350**, *P=0.136* , P=0.002	P=0.726
LV Mass Cor mg	93.38 ± 1.06	92.30 ± 5.69	114.78 ± 4.42	127.89 ± 7.42	104.24 ± 8.59	108.86 ± 9.68	P>0.999, **P=0.350**, *P=0.136* , P=0.002	P=0.726
LVAW;s mm	1.15 ± 0.05	1.14 ± 0.05	1.28 ± 0.03	1.39 ± 0.03	1.15 ± 0.04	1.10 ± 0.05	P=0.999, **P=0.104**, *P=0.093* , P<0.001	P=0.459
LVAW;d mm	0.89 ± 0.02	0.85 ± 0.04	0.97 ± 0.04	1.07 ± 0.04	0.89 ± 0.03	0.89 ± 0.04	P=0.947, **P=0.281**, *P=0.568* , P=0.004	P=0.933
LVPW;s mm	1.10 ± 0.02	1.12 ± 0.04	1.29 ± 0.07	1.30 ± 0.05	1.18 ± 0.09	1.16 ± 0.06	P=0.997, **P>0.999**, *P=0.089* , P=0.143	P=0.887
LVPW;d mm	0.80 ± 0.03	0.84 ± 0.02	0.86 ± 0.05	0.89 ± 0.04	0.80 ± 0.05	0.81 ± 0.03	P=0.963, **P=0.985**, *P=0.779* , P=0.917	#P=0.463
Left Atrial Area mm^2	4.04 ± 0.27	4.08 ± 0.31	4.07 ± 0.21	4.09 ± 0.17	4.07 ± 0.36	3.84 ± 0.27	P>0.999, **P>0.999**, *P>0.999* , P>0.999	P=0.620

Definition of abbreviations: A' = peak velocity of diastolic mitral annular motion; AET = aortic ejection time; E' = peak velocity of early diastolic mitral annular motion; IVCT = isovolumic contraction time; IVRT = isovolumic relaxation time; MV = mitral valve; A = peak velocity of late transmitral flow; E = peak velocity of early diastolic transmitral flow; LV = left ventricle; MPI = myocardial performance index; s = systolic; d = diastolic; LVAW = left ventricle anterior wall; LVPW = left ventricle posterior wall. Values are mean ± SEM using a one way ANOVA for WT AA and SS mice. P values are in normal font for AA vs. AA + febuxostat, **bolded** for SS vs. SS + febuxostat, *italicized* for AA vs. SS, and underlined for AA + febuxostat vs. SS + febuxostat. Values are mean ± SEM using a students t test for *Xdh* mice unless otherwise noted. +Values are mean ± SEM using an unpaired t test with Welch's correction. #Values are mean ± SEM using a Mann-Whitney test.

Supplemental Table VIII. Hepatocyte-specific XO knockout significantly decreased uric acid concentration and XO activity in plasma and liver tissue of H*Xdh* -/- mice at 10 weeks post-engraftment with effects on plasma detected as early as 3 weeks.

		0 week		3 week		10 week		Significance
		$XDH^{fl/fl}$ *(n=8)*	$XDH^{-/-}$ *(n=6-7)*	$XDH^{fl/fl}$ *(n=8)*	$XDH^{-/-}$ *(n=6-7)*	$XDH^{fl/fl}$ *(n=7-8)*	$XDH^{-/-}$ *(n=6-7)*	$XDH^{fl/fl}$ 0 vs. $XDH^{-/-}$ 0 **$XDH^{fl/fl}$ 3 vs. $XDH^{-/-}$ 3** $XDH^{fl/fl}$ 10 vs. $XDH^{-/-}$ 10
Plasma	Uric Acid, µM	1.52 ± 0.16	1.16 ± 0.10	1.88 ± 0.16	1.05 ± 0.09	1.00 ± 0.14	0.31 ± 0.05	P=0.231, **P=0.003** P=0.005
	XO Activity, µUnits/mL	91.21 ± 9.70	72.82 ± 6.07	112.83 ± 9.49	68.26 ± 2.01	229.40 ± 33.13	70.74 ± 12.27	P=0.355, **P=0.006** P=0.005
Liver	Uric Acid, µM	-	-	-	-	93.44 ± 1.44	14.84 ± 1.11	P<0.001
	XO Activity, mUnits/mg	-	-	-	-	24.56 ± 1.47	4.13 ± 0.43	#P<0.001
Lung	Uric Acid, µM	-	-	-	-	20.46 ± 2.78	20.70 ± 1.76	P=0.946
	XO Activity, mUnits/mg	-	-	-	-	12.67 ± 1.88	13.92 ± 0.87	P=0.575
Kidney	Uric Acid, µM	-	-	-	-	15.31 ± 0.79	12.29 ± 2.54	+P=0.294
	XO Activity, mUnits/mg	-	-	-	-	4.87 ± 0.22	3.82 ± 0.78	+P=0.236

Definition of abbreviations: XO = xanthine oxidase; - = measurement not made. Values are mean ± SEM using a two-way ANOVA with Sidak's multiple comparison test for plasma measurements. Values are mean ± SEM using an unpaired t-test unless otherwise noted. #Values are mean ± SEM using a Mann-Whitney test. +Values are mean ± SEM using an unpaired t test with Welch's correction. P values are in normal font for *Xdh* fl/fl 0 week vs. H*Xdh* -/- 0 week, **bolded** for *Xdh* fl/fl 3 week vs. H*Xdh* -/- 3 week, and underlined for *Xdh* fl/fl 10 week vs. H*Xdh* -/- 10 week.

Supplemental Table IX. Blood cell indices for Townes SS bone marrow transplanted *Xdh* fl/fl and H*Xdh* -/- chimeras at weeks 0, 3, 6, and 10 weeks post-engraftment.

	0 week		3 week		6 week		10 week		Significance
	SS + XDH fl/fl *(n=8)*	SS + XDH -/- *(n=7)*	SS + XDH fl/fl *(n=7-8)*	SS + XDH -/- *(n=7)*	SS + XDH fl/fl *(n=8)*	SS + XDH -/- *(n=7)*	SS + XDH fl/fl *(n=8)*	SS + XDH -/- *(n=7)*	SS + XDH fl/fl 0 vs SS + XDH -/- 0 **SS + XDH fl/fl 3 vs SS + XDH -/- 3** *SS + XDH fl/fl 6 vs SS + XDH -/- 6* SS + XDH fl/fl 10 vs. SS + XDH -/- 10
WBC, 10^3/µL	16.03 ± 1.19	20.44 ± 0.98	20.13 ± 1.05	22.04 ± 1.34	22.74 ± 1.57	25.17 ± 1.23	27.16 ± 0.54	30.76 ± 2.30	P=0.095, **P=0.805** *P=0.605,* P=0.263
RBC, 10^6/µL	6.27 ± 0.15	6.23 ± 0.11	5.79 ± 0.08	6.04 ± 0.15	5.55 ± 0.16	6.03 ± 0.17	5.90 ± 0.22	5.73 ± 0.12	P>0.999, **P=0.681** *P=0.105,* P=0.898
HCT, %	30.54 ± 0.58	30.34 ± 0.61	27.25 ± 0.31	28.47 ± 0.92	26.19 ± 0.79	28.21 ± 0.81	27.14 ± 0.98	26.26 ± 0.69	P>0.999, **P=0.680** *P=0.212,* P=0.873
MCV, fl	48.75 ± 0.44	48.73 ± 0.41	47.09 ± 0.46	47.09 ± 0.57	47.23 ± 0.47	46.73 ± 0.39	45.99 ± 0.47	45.80 ± 0.45	P>0.999, **P>0.999** *P=0.909,* P=0.997
RDW, %	34.23 ± 0.42	34.01 ± 0.36	36.39 ± 0.58	37.01 ± 1.08	36.35 ± 0.42	36.56 ± 0.46	36.81 ± 0.62	36.89 ± 0.46	P=0.998, **P=0.908** *P=0.999,* P>0.999
HGB, g/dL	10.54 ± 0.19	10.56 ± 0.16	9.74 ± 0.11	10.13 ± 0.24	9.275 ± 0.24	9.96 ± 0.26	9.56 ± 0.27	9.47 ± 0.20	P>0.999, **P=0.602** *P=0.114,* P=0.997
MCH, pg	16.86 ± 0.19	16.94 ± 0.16	16.84 ± 0.19	16.79 ± 0.17	16.75 ± 0.15	16.54 ± 0.17	16.26 ± 0.20	16.56 ± 0.13	P=0.996, **P=0.999** *P=0.872,* P=0.657
PLT, 10^3/µL	464.00 ± 18.74	425.71 ± 8.25	476.63 ± 19.25	511.14 ± 34.93	444.75 ± 24.52	515.00 ± 16.41	489.00 ± 30.01	511.57 ± 25.23	P=0.695, **P=0.769** *P=0.152,* P=0.939
MPV, fl	6.25 ± 0.04	6.24 ± 0.04	6.33 ± 0.06	6.34 ± 0.05	6.38 ± 0.07	6.33 ± 0.03	6.41 + 0.07	6.34 ± 0.05	P>0.999, **P=0.999** *P=0.957,* P=0.837
Retic. %	47.75 ± 1.15	47.83 ± 0.69	49.50 ± 1.10	47.90 ± 1.43	43.89 ± 0.82	42.33 ± 1.21	47.50 ± 1.19	47.60 ± 0.49	P>0.999, **P=0.747** *P=0.764,* P>0.999

Definition of abbreviations: WBC = white blood cells; RBC = red blood cells; HCT = hematocrit; MCV = mean corpuscular volume; RDW = red blood cell distribution width; HGB = hemoglobin; MCH = mean corpuscular hemoglobin; PLT = platelets; MPV = mean platelet volume; Retic = reticulocyte. Values are mean ± SEM using a two-way ANOVA with Sidak's multiple comparison test. P values are in normal font for SS *Xdh* fl/fl 0 week vs. SS + H*Xdh* -/- 0 week, **bolded** for SS + *Xdh* fl/fl 3 week vs. SS + H*Xdh* -/- 3 week, *italicized* for SS + *Xdh* fl/fl 6 week vs. SS + H*Xdh* -/- 6 week, and underlined for SS + *Xdh* fl/fl 10 week vs. SS + H*Xdh* -/- 10 week.